The Variable Metabolic Response to Dietary Isoflavones in Humans (43829)

G. E. KELLY,*[,1] G. E. JOANNOU,† A. Y. REEDER,† C. NELSON,* AND M. A. WARING*

Department of Surgery, University of Sydney, New South Wales 2006 and Department of Metabolic Mass Spectrometry,† Royal Prince Alfred Hospital, New South Wales, Australia*

Abstract. **The aim of this study was to better understand the metabolic fate of dietary estrogenic isoflavones in humans. Twelve volunteers were challenged with soya flour and urinary levels of the isoflavones daidzein (Da), genistein (Gen), and glycitein (Gly), and the isoflavonoid metabolites equol (Eq) and O-desmethylangolensin (O-Dma) determined by GC and GC-MS. Prior to challenge, Da, Gen, and Gly were present in the urine of all participants at low levels and Eq and O-Dma were present in 9/12 and 10/12 participants, respectively. Urinary levels of all five diphenols were increased in each individual on the day following challenge, returning to approximately prechallenge levels on the second or third day post-challenge. Mean post-challenge peak levels of the five diphenols compared with pre-challenge levels were Da (4×), Gen (8×), Gly (5×), Eq (45×), and O-Dma (66×). However, there was considerable individual variation in this metabolic response with peak levels of Eq showing the highest variation (1527×). An inverse relationship between Eq and O-Dma excretion was also found post-challenge suggesting individual variability in the preferred metabolic pathways of dietary isoflavones.** [P.S.E.B.M. 1995, Vol 208]

The recently described presence in human urine of diphenols of plant origin has excited scientific attention because of these diphenols' range of biological activities, which include antiangiogenic (1), antiestrogenic (2), and enzyme-inhibitory (3, 4) effects. The substances also are thought to have anticancer (5, 6) effects.

The two principal classes of plant-derived diphenols are isoflavones and lignans. In each case, the parent plant compounds undergo fermentation by bowel microflora, with both metabolites and unfermented parent (aglycone) compounds being liable to absorption. The parent compounds are reconjugated to glucuronides in the body, but otherwise are thought not to undergo any further metabolism in the body, and are excreted in the urine (7).

The parent estrogenic isoflavones daidzein (Da), genistein (Gen), formononetin (For), and biochanin A (BA) occur almost exclusively in legumes (8), while lignans such as metairesinol occur widely in fruits, vegetables, and cereals (9).

The metabolism of estrogenic isoflavones and lignans is poorly understood in humans. Following ingestion of isoflavone-rich foods such as soya, the following isoflavones have been described in human urine (10–17): Da and Gen; the Da metabolites—equol (Eq) and O-desmethylangolensin (O-Dma); an intermediate compound between Da and O-Dma (Intermediate O); and an intermediate compound between Da and Eq (Intermediate E). The lignan metabolites detected in human urine following ingestion of cereals and vegetables are enterodiol and enterolactone (11, 17).

The levels of urinary Eq following ingestion of a standard amount of dietary isoflavones also has been reported to be highly variable between individuals (up to 400×) (10, 17), suggesting variable metabolic pathways or metabolic capacity.

The following study is an attempt to better understand the metabolic response of humans to dietary isoflavones.

Materials and Methods

The participants were 12 (6 male, 6 female) healthy Caucasian volunteers aged between 25 and 51 years; all women were premenopausal; none of the participants were taking medication (including oral contra-

[1] To whom requests for reprints should be addressed at Department of Surgery, University of Sydney, New South Wales 2006, Australia.

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ception) and none had received antibiotic therapy in the 6 months prior to the study; all participants were nonsmokers. Participants were fed a legume-free diet for 1 week and then challenged with 40 g of whole soya flour daily for 2 days. Twenty-four-hour urine samples were collected from each participant on the day prior to and on the 3 days immediately following the second day soya challenge. All daily urine samples were analyzed separately.

The methods of isolation of the urinary diphenols and their characterization by GC and GC-MS are described in detail elsewhere (18). Reference standards supplied by others were Eq, Da, Gen, and dihydrodaidzein. O-Dma and enterolactone were identified by comparison with published data (15, 19–21).

Results

The soya flour was analyzed and found to contain 980 μg genistein, 800 μg daidzein and 30 μg glycitein/g. Therefore, each individual received approximately 78 mg Gen, 64 mg Da, and 24 mg Gly per challenge over 2 days.

The urinary levels of isoflavones, isoflavonoid metabolites, and enterolactone both before and following soya challenge are summarised in Table I.

Isoflavones. Prior to the soya challenge, Gen, Da, and Gly were present in the urine of all 12 individuals. Following the soya challenge, each subject showed elevated urinary levels of all three isoflavones. In each case, urinary levels were greatest on the first day post-challenge, returning to baseline or near-baseline levels by the second or third day post-challenge.

Isoflavonoid Metabolites. The limits of detectability of Eq and O-Dma in this study were determined to be approximately 0.02 μmol.

Eq was detected in the urine of 9/12 individuals pre-challenge and in all 12 individuals post-challenge. In 11/12 individuals, there was a sharp rise in Eq levels post-challenge; in each case, the levels peaked on the first day post-challenge and thereafter fell slowly to still be in each case well above prechallenge levels on the third day post-challenge. The remaining individual had no detectable Eq levels prechallenge and showed a barely detectable (0.04 μmol) post-challenge rise.

O-Dma was detected in the urine of 10/12 individuals prechallenge and in all 12 individuals post-challenge. In each case, there was a sharp rise in O-Dma levels post-challenge with levels peaking on the first day post-challenge and thereafter falling slowly; in 10/12 individuals, O-Dma levels on the third day post-challenge remained at least four times pre-challenge levels.

Enterolactone. Enterolactone was present in the urine of all 12 individuals both before and following the soya challenge; enterolactone levels were unaffected by the challenge.

Interrelationship of Urinary Diphenols. In a separate analysis, individuals were grouped according to their Eq excretion rates (high versus low) over the three post-challenge days, and the relationship between the excretion of Eq and those of Da and O-Dma compared. This data is summarised in Table II. A higher rate of Eq excretion appeared to be associated with lower rates of excretion of Da (r = 0.5) and O-Dma (r = 0.3).

Discussion

This study confirms that soya is a rich source of the isoflavones, Da and Gen. Following ingestion, these dietary isoflavones undergo intestinal metabo-

Table I. Mean (Range) of Daily Urinary Levels of Phytoestrogens and Some of Their Metabolites Immediately Prior to and in a 3-Day Period Immediately Following a Soya Challenge

	Prechallenge (μmol)	Days post-challenge (μmol)			
		Day 1	Day 2	Day 3	3-Day total
Isoflavones					
Daidzein	3.8 (1.8–6.6)	14.7 (7.3–25.6)	4.3 (0.1–14.9)	2.1 (0.4–6.3)	20.3 (9.6–40.6)
Genistein	1.1 (0.8–2.0)	8.4 (2.7–19.6)	1.5 (0.2–4.8)	1.4 (0.1–4.8)	11.2 (3.5–23.7)
Glycitein	0.8 (0.02–2.4)	3.6 (0.6–6.4)	0.3 (0.02–0.9)	1.0 (0.1–3.5)	4.0 (0.6–7.5)
Isoflavonoid Metabolites					
Equol	0.2 (ND[a]–0.7)	9.1 (0.04–61.1)	5.8 (0.02–56.5)	3.5 (0.02–34.7)	22.6 (0.2–152.3)
0-Dma	0.2 (ND–0.5)	13.2 (2.3–44.4)	2.7 (0.2–12.7)	1.3 (0.3–3.3)	14.4 (3.4–58.9)
Lignan					
Enterolactone	11.0 (0.9–35.3)	9.3 (7.5–26.9)	7.2 (1.9–22.6)	11.9 (2.5–20.3)	24.0 (2.5–73.1)

[a] ND = not detected.

Table II. Comparison of the Urinary Excretion Rates of Daidzein and Two Daidzein Metabolites in Individuals over a 3-Day Period Following Soya Challenge[a]

Metabolite	Mean (SD) excretion (μmol) per 3 days	
	<8 μmol equol (n = 8)	>25 μmol equol (n = 4)
Daidzein	23.05 (12.43)	14.95 (6.69)
Equol	1.53 (2.60)	64.89 (59.23)
O-Dma	21.72 (17.93)	6.97 (6.47)

[a] Individuals are grouped as either low equol-producers (less than 8 μmol in 3 days) or high equol-producers (over 25 μmol in 3 days).

lism leading to the absorption and excretion in the urine of both the parent compounds and some of the intestinal metabolites. The mean peak urinary levels post-challenge versus prechallenge for Da, Gen, Eq, and O-Dma were 4×, 8×, 45×, and 66×, respectively.

All individuals showed slightly greater urinary excretion of Gly in line with the moderately low levels present in the soya, but there was no overall increase in the excretion of enterolactone, suggesting no greater lignan levels in the soya compared with the background diet.

The results of this study lead to a number of comments on Da and Gen metabolism in humans. The first comment is that overall, higher levels of Da compared with Gen were recovered in the urine despite the soya containing higher levels of genistein:daidzein. Whether or not this reflects an overall lesser ability in humans to assimilate intact Gen or a greater susceptibility of Gen to intestinal fermentation is not clear from this study.

The second comment is on the half-life of the urinary diphenols. The parent Da and Gen compounds have a substantially shorter half-life than the isoflavonoid metabolites, Eq and O-Dma. Whereas urinary Da and Gen levels generally had returned to normal baseline levels by Day 2 or 3 post-challenge, mean urinary Eq and O-Dma levels were still at least five times baseline levels by the third day post-challenge.

The third comment is the high individual variability of metabolic response to Da and Gen. Although all individuals in this study received a standard dose of the isoflavones, there were substantial variations between individuals in the urinary excretion rates of the diphenols. When the total excretion of diphenols over the 3 days post-challenge was considered, the individual variability for Da, Gen, Eq, and O-Dma 4×, 7×, 761×, and 17× respectively; peak Eq levels varied 1527× between individuals (Table I).

These results indicate that all 12 individuals had the ability to ferment the dietary isoflavones in the gut and to assimilate both the parent isoflavones and their metabolites, however they also point to a highly variable metabolic capacity and/or differentially active metabolic pathways. The latter is supported by the finding that there was an overall indirect relationship between urinary excretion rates of Eq and O-Dma, suggesting at least in the case of Da, variable activities of two alternate pathways of metabolism (Table II). This latter finding may have implications for the purported physiological significance of dietary isoflavones (6), given that Eq is known to be substantially more estrogenic compared to both Da and O-Dma (8).

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